U.S. Wheat and Barley Scab Initiative FY00 Final Performance Report (approx. May 00 – April 01) July 30, 2001

Cover Page

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| Year: | FY2000 (approx. May 00 – April 01) |
| Grant Number: | |
| Grant Title: | Fusarium Head Blight Research |
| 2000 ARS Award Amount: | \$48,780 |

Project

| Program Area | Project Title | Requested Amount |
|---------------|--|--------------------------|
| Biotechnology | Spike-specific gene promoter isolation | \$50,348.00 |
| | from Bowman barley and near-isogenic | |
| | morphological marker lines. | |
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| | | |
| | Requested Total | \$50,348.00 ¹ |

Principal Investigator

Date

¹ Note: The Requested Total and the Award Amount are not equal.

Project 1: Spike-specific gene promoter isolation from Bowman barley and near-isogenic morphological marker lines.

1. What major problem or issue is being resolved and how are you resolving it?

Barley transformation has the potential to help combat Fusarium head blight by introducing anti-fungal and anti-toxin genes. Promoters currently in use for barley transformation give transgene expression in all plant tissue throughout development, which is not an efficient use of plant resources. This project will isolate spike-specific promoters to target gene expression to the spike tissues that are attacked by Fusarium head blight. RNA differential display technology is being used to identify genes that are expressed in spike tissues of Bowman and ten near-isogenic lines with morphological mutations in spike tissues. Regulatory regions, i.e. promoters, of these genes will be identified by DNA sequencing and 5'-rapid amplification of cDNA ends (5'RACE). Candidate spike-specific promoters will be inserted into marker gene constructs and tested for transgene expression patterns. Differential display comparisons include Bowman spike tissue vs. non-spike tissue, expressing vs. non-expressing tissue of the morphological marker lines, and morphological line tissue expressing the trait vs. the comparable normal tissue in Bowman.

2. What were the most significant accomplishments?

Potential differentially expressed sequences have been identified from comparisons between seven of the morphological marker lines and Bowman. Sequences isolated include both those that were found in tissue expressing the morphological traits but not in normal tissue, and those found in spikes of mutant and normal plants but not in leaf tissue of the same plants. Sequences from three of the populations have been reamplified, cloned, and dot blotted for confirmation of differential expression. These include 84 sequences from rob1.a (orange lemma), 95 from gsh2.f (glossy sheath and spike), and 58 from cer-i.16 (glossy spike). Hybridization of the dot blots with cDNA from leaf and spike tissues is underway to confirm which of these sequences are actually differentially expressed in spikes. Sequences from glo-a.1003 (globosum), Gle1.a (glossy lemma and pericarp), alm1.a (albino lemma) and gsh6.s (glossy sheath and spike) have been isolated from gels and are being prepared for reamplification. Differential display reactions for the yhd1.a (yellow head) population are being completed. Additional RNA extractions are needed from the last two morphological lines, Pre2.b (red lemma and pericarp) and Blp1.b (black lemma and pericarp), before differential display reactions can be completed. Analysis of these two morphological lines is on hold while experiments are being completed on the other seven lines.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Dahleen, L.S., P.A. Okubara and A.E. Blechl. 2001. Transgenic approaches to combat Fusarium head blight in wheat and barley. Crop Sci. 40:628-637.

Manoharan, M., T.M. Hohn and L.S. Dahleen. Genetic transformation of barley with genes for scab resistance. National Fusarium Head Blight Forum Proceedings. 2000.